BIO-RAD

REF 17001379

ddPCR™ Dx Universal Kit for QX200™ ddPCR™ Dx System

Instructions For Use

Σ 192

CE

IVD

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TRANSLATIONS

Product documents may be provided in additional languages on electronic media.

SYMBOLS LEXICON

CE European Conformity	 Manufacturer	EC REP Authorized Representative in the European Union
LOT Lot Number	 Use by	IVD For In Vitro Diagnostic Use
 Temperature Limit	REF Catalog Number	 Consult Instructions for Use
 Number of Tests	USE For use with	SN Serial Number
Rx Only Precription Use Only	 Contains Latex	

BIO-RAD TECHNICAL SUPPORT

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SAFETY AND REGULATORY COMPLIANCE

QX200™ System has been tested and found to be in compliance with all applicable requirements of the following safety and electromagnetic standards:

1. IEC 61010-1:2010 (3rd ed.), EN61010-1:2010 (3rd ed). Electrical Equipment for Measurement, Control, and Laboratory Use — Part 1: General requirements
2. EN 61326-1:2006 (Class A). Electrical equipment for measurement, control, and laboratory use. EMC requirements, Part 1: General requirements
3. UL 61010-1:2004, Laboratory equipment, Test & Measurement Equipment and Industrial Process Controls
4. CAN/CSA 22.2 No 61010-1-04, Safety Requirements for Electrical. Equipment for Measurement, Control, and Laboratory Use, Part I: General. Requirements
5. This equipment generates, uses, and can radiate radiofrequency energy and, if not installed and used in accordance with the instruction manual, may cause harmful interference to radio communications. Operation of this equipment in a residential area is likely to cause harmful interference, in which case the user will be required to correct the interference at his own expense

CE The CE mark indicates that the manufacturer ensures the product conforms with the essential requirements of the European Directive for in-vitro diagnostic medical devices 98/79/EC.

The CSA mark indicates that a product has been tested to Canadian and U.S. standards, and it meets the requirements of those applicable standards.

This equipment has been tested and found to comply with the limits for a Class A digital device pursuant to Part 15 of the FCC Rules. These limits are designed to provide reasonable protection against harmful interference when the equipment is operated in a commercial environment.

The Waste Electrical and Electronic Equipment Directive symbol indicates that when the end- user wishes to discard this product, it must be sent to separate collection facilities for recovery and recycling.

This instrument is for use only by trained personnel.

Do not position the equipment so that it is difficult to operate the plug of the power supply. The plug of the power supply is the disconnect device.

No serviceable parts inside.

ddPCR™ Dx Universal Kit for QX200™ System Warnings and Precautions

For in vitro diagnostic use. For healthcare professional use.

This test kit should be handled only by qualified personnel trained in laboratory procedures and familiar with their potential hazards. Wear appropriate protective clothing, gloves and eye/face protection and handle appropriately with the requisite Good Laboratory Practices.

PPE (Personal Protective Equipment) Training

Proper use of gloves is recommended with use of oils and sample plates. OSHA requirements for PPE are set forth in the Code of Federal Regulations (CFR) at 29 CFR 1910.132 (General requirements); 29 CFR 1910.138 (Hand protection); 29 CFR 1926.95 (Criteria for standard personal protective equipment). Any gloves with impaired protective ability should be discarded and replaced. Consider the toxicity of the chemicals and factors such as duration of exposure, storage, and temperature when deciding to reuse chemically exposed gloves. Features to aid glove selection for handling of machines, assays, oils, and cleaning solvents:

6. Butyl gloves are made of a synthetic rubber and protect against peroxide, hydro uoric acid, strong bases, alcohols, aldehydes, and ketones
7. Natural (latex) rubber gloves are comfortable to wear and feature outstanding tensile strength, elasticity, and temperature resistance
8. Neoprene gloves are made of synthetic rubber and offer good pliability, nger dexterity, high density, and tear resistance; they protect against alcohols, organic acids, and alkalis
9. Nitrile gloves are made of copolymer and provide protection from chlorinated solvents such as trichloroethylene and tetrachloroethene; they offer protection when working with oils, greases, acids, and caustic substances.

Table of Contents

Translations ii
Symbols Lexicon ii
Bio-Rad Technical Support ii
Legal Notices ii
Safety and Regulatory Compliance iii
ddPCR™ Dx Universal Kit for QX200™ System Warnings and Precautions iv
PPE (Personal Protective Equipment) Training iv
In Vitro Diagnostic Medical Device 3
Principle of Procedure 3
Reagents & Supplies 4
Materials Provided 4
Materials required but not provided 5
Storage and Stability 5
Warnings and Precautions 6
Quality Control 6
Specimen Collection 6
Sample & Reaction Preparation 6
DNA Digestion 6
DNA Digestion in ddPCR Reaction 7
DNA Digestion Prior to ddPCR Reaction 7
Procedure 7
Workflow 7
Reaction Preparation & Setup 7
Manual Droplet Generation 8
Thermal Cycling 9
Data Acquisition and Analysis 10

In Vitro Diagnostic Medical Device

Common Name: **Dx Universal Kit for QX200™ System**

Name: **Dx Universal Kit for QX200™ ddPCR™ Dx System**

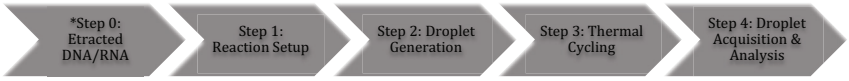
p/n: **17001379**

INTENDED USE

The ddPCR Dx Universal Kit for QX200 ddPCR Dx System (p/n: 17001379) is a set of non-analyte-specific reagents and consumables required to be used on QX200 Droplet Digital PCR System in order to generate and read droplets. The end user supplies the diagnostic assay, controls and calibrators.

PRINCIPLE OF PROCEDURE

The ddPCR DX Universal Kit for QX200 ddPCR Dx System, is a kit comprising of reagents and consumable needed to run a ddPCR reaction workflow on the QX200 system, except primers, probe(s), and template.



*Step 0 is needed should there be a need to convert RNA into cDNA.

The Dx Universal Kit is designed and packaged to simplify and enable the use of QX200 ddPCR System in open mode. Allowing the laboratory to develop an assay of interest without the need to figure out common consumables necessary for the instrument and the technology. Thus enabling the lab to focus more on designing their primers, probe(s) and template sequences.

The kit consists of a ddPCR Dx Consumable Pack, ddPCR Dx Supermix Pack, and ddPCR Droplet Reader Oil Pack.

REAGENTS & SUPPLIES

Materials Provided

Σ	***NOTE:*** *Catalog # below (120001921, 12002544 and 12002526) all are included in ddPCR Dx Universal Kit for QX200 ddPCR Dx System*

Catalog Number	Component	Quantity	Description/Presentation
17001379	**ddPCR Dx Universal Kit for QX200 ddPCR Dx System**	**192 Rxns**	
12003185	ddPCR 96-Well Plates	1x 5	5 Ready for Use 96-well plates
12003015	ddPCR Pierceable Foil Heat Seal	1 x 50	1 bag of 50 heat sales. Ready for use
12003020	Droplet Generation Oil for Probes	2 x 7 ml	2-7mL bottles for oil. Ready for use
12003018	DG8™ Cartridges	1 x 24	1-24 count package of droplet generation cartridges. Ready for use
12003021	DG8 Gaskets	1 x 24	1-24 count package of seals. Ready for use
12005493	Instructions Manual	1	Instructions for Use for ddPCR Dx Consumable Pack

Catalog Number	Component	Quantity	Volume
12002544	**ddPCR Dx Supermix Pack**	**2**	**1 mL**

Thaw to room temperature before using Supermix for manual QX200 System.

Catalog Number	Component	Quantity	Volume
12002526	**QXDx Droplet Reader Oil Pack**	**1**	**1 L**

Ready to use Droplet Reader Oil for use with QX200 System.

Materials required but not provided

Description	Catalog #
QX200 Dx Digital Droplet PCR System	**17000034**
QX200 Droplet Reader, IVD • QuantaSoft Software • Plate Holders (2) • USB 2.0 cable • Power Cord	12001045 10026368 29711024 Call technical support Call technical support
QX200 Droplet Generator, IVD • Cartridge Holder • Power Cord	12001049 186-3051 Call technical support

Description	Catalog #
Pipets	
• 20 µL pipet for sample loading • 50 µL pipet for droplet transfer • 8-channel, 200 µL pipet for oil	Rainin L-20 Rainin L8-50 Rainin L8-200

Description	Catalog #
Pipet Tip (filtered)	Rainin GP-L10F GP-L200F

Description
Required: Thermal Cyclers with Specifications equivalent to the following: • Accuracy: +/- 0.2°C • Uniformity: +/- 0.4°CC well-to-well within 10 sec • Adjustable ramp capability with required ramp rate: up to 2°C/sec • Temperature range: 0-100°C

Description	Catalog #
PX1™ PCR Plate Sealer	**181-4000**

STORAGE AND STABILITY

ddPCR Dx Supermix Pack is stable at -25°C to -15°C through the stated expiration date printed on the labels. Once thawed, the supermix can be stored at 4°C for up to 2 weeks. Repeated freezing and thawing of the supermix is not recommended.

ddPCR Dx Consumable Pack is stable at 15-30°C (room temperature) through the stated expiration date printed on the labels.

ddPCR Dx Droplet Reader Oil Pack is stable at a constant temperature of 15-30°C (room temperature) through the stated expiration date printed on the labels.

Warnings and Precautions

- This product is For Laboratory Use
- Do not use expired materials
- Always handle specimens in accordance with safe laboratory procedures such CLSI Document M29[1]
- Protect reagents against heat and humidity
- Wear personal protective equipment, while handling all reagents. Wash hands thoroughly after performing the test.
- Dispose

Quality Control

ddPCR Dx Supermix for Probes (No dUTP) is free of contaminating DNase and RNase.

Stringent specifications are maintained to ensure lot-to-lot consistency.

SPECIMEN COLLECTION

- Collect specimens and label appropriately
- Collected specimens should be maintained per the conditions validated by the user
- For best practices, collect whole blood samples according to standard techniques at each institution. Collect a minimum of 5 mL blood specimens in tubes containing EDTA as anticoagulant.
- Proceed to sample and reaction preparation

SAMPLE & REACTION PREPARATION

Sample Extraction is required. Input to ddPCR workflow is extracted DNA or cDNA.

Application specific extraction preparation procedures should be developed and validated by the user.

- Common sample preparation methodologies for extraction of DNA from whole blood or buffy coat are compatible with ddPCR methods.
- Common sample preparation methodologies for extraction of DNA from FFPE tissue are compatible with ddPCR methods.
- For best practices, at least 10 million ($1x10^7$) nucleated cells should be collected and extracted.

  Recommendation for Optimal Results:

  - Follow general guidelines and recommendations for QX200 ddPCR System manual
  - The concentration of human genomic DNA should be 30 pg ~330 ng per 20 µL reaction digested.

DNA Digestion

DNA fragmentation by restriction digestion prior to droplet generation enables optimal accuracy by separating tandem gene copies, reducing sample viscosity, and improving template accessibility for input samples. Restriction digestion improves overall performance of digital PCR across applications.

Recommendation: Choose a restriction endonuclease that does not cut either the target or reference amplicon and that is insensitive to methylation. Four-base cutters and high-fidelity enzymes are preferred.

Two Strategies may be used to perform restriction digestion of DNA samples: digestion directly in the ddPCR reaction during setup, or conventional digestion prior to ddPCR.

DNA Digestion in ddPCR Reaction

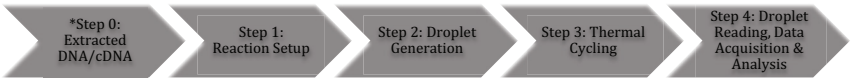
- Efficient digestion of sample DNA can be achieved by direct addition of restriction enzymes to the ddPCR reaction.
- Dilute the restriction enzyme using the recommended diluent buffer according to the manufacturer's instructions, and 1 µL to the ddPCR reaction.
- Approximately 2-5 units of restriction enzyme per 20 µL ddPCR reaction are recommended.
- The addition of restriction enzyme buffer with high salt can inhibit digital PCR and should be avoided.
- HaeIII, MseI, AluI, HindIIII, and CviQI have been observed to work well for digestions in ddPCR reactions.

DNA Digestion Prior to ddPCR Reaction

- Restriction Enzyme digestion can be carried out as a separate reaction before ddPCR reaction setup.
- Use 5-10 enzyme units per microgram DNA, and 10-20 enzyme units per microgram genomic DNA.
- Incubate the reaction for 1hr at the temperature recommended for the restriction enzyme.
- Heat inactivation is not required, but can be considered if long-term storage is required; do not heat inactivate above 65°C.
- DNA purification is not necessary after restriction digestion.
- Use a minimum of 10-fold dilution of the digest to reduce the salt content of the sample in ddPCR reaction.
- Store digested DNA at -20°C or below.
- Prepare the ddPCR reaction mix without the diluted restriction enzyme.

PROCEDURE

Workflow



*Step 0 is needed should there be a need to convert RNA into cDNA.

Reaction Preparation & Setup

1. Thaw all supermix, primers and probes components to room temperature. Mix thoroughly by vortexing the tube to ensure homogeneity because a concentration gradient may form during –20°C storage. Centrifuge briefly to collect contents at the bottom of the tube.
2. Prepare samples at the desired concentration before setting up the reaction mix.
3. Prepare the reaction mix for the number of reactions needed.

4. Assemble all required components except the sample, dispense equal aliquots into each reaction tube, and add the sample to each reaction tube as the final step.
5. Mix thoroughly by vortexing the tube. Centrifuge briefly to ensure that all components are at the bottom of the reaction tube. Allow reaction tubes to equilibrate at room temperature for about 3 minutes.

Component	Volume per Reaction (µL)	Final Concentration
ddPCR Supermix for Probes (No dUTP)	12.5	1x
20x target primers/probe (FAM)	1.25	900 nM/250 nM
20x reference primers/probe (HEX/VIC)	1.25	900 nM/250 nM
Diluted restriction enzyme (see DNA Digestion)	1	Variable
Sample	Variable	Up to 330 ng*
RNase & DNase Free Water	Variable	-
Total Volume	25	-

Table 1

* Sample concentrations >66ng per reaction and certain applications may require restriction digestion for optimal target detection. If digestion is not required, prepare the ddPCR reaction mix without the diluted restriction enzyme.

Upon completion the reaction preparation & setup, move forward to step 2 – Droplet Generation workflow.

Manual Droplet Generation

Refer to the QX200 Dx Digital Droplet PCR System Instruction Manual (REF 10000046997) for detailed graphic driven setup & use instructions.

QX200 Droplet Generator, IVD prepares droplets for up to 8 samples at a time. Droplet generation takes ~2 minutes for each set of eight samples (~30 minutes for a 96 well plate)

NOTE: *All 8 wells in the DG8 Droplet generator cartridge must contain sample or no template control, and all 8 wells must contain droplet generation oil.*

Upon completion of droplet generation, from QX200 Droplet Generator, carefully transfer the droplets into a clean 96-well plate and seal the 96-well plate containing the droplets with a PX1 PCR Plate Sealer. Remove the droplet plate containing ddPCR droplets and seal within 30 min of droplet generation completing.

Follow the instructions in the PX1 PCR Plate Sealer:

- Set the plate sealer temperature to 180°C and time to 5 sec.
- Touch the arrow to open the PX1 tray door. Position the support block on the tray with the 96-well side facing up. Place the 96-well plate onto the support block and ensure that all plate wells are aligned with the support block.
- Cover the 96-well plate with one sheet of pierceable foil seal. (The yellow label on the Bio-Rad heat seal bag identifies the sealing surface.) Do not attempt to place the frame over the foil-covered plate. The frame is only for use with other seals.
- Once the 96-well plate is secured on the support block and covered with the pierceable foil seal, touch the Seal button. The tray will close and heat sealing will initiate.
- When heat sealing is complete, the PX1 door will open automatically. Remove the plate from the block for thermal cycling. Remove the block from the PX1 Sealer.
- Check that all the wells in the plate are sealed; the depressions of the wells should be visible on the foil. Once sealed, the plate is ready for thermal cycling.

Thermal Cycling

Once the 96-well plate containing the droplets is sealed, place it into the thermal cycler for PCR amplification.

Cycling Step	Temperature (°C)	Time	Ramp Rate	Number of Cycles
Enzyme Activation	95	10 min	2 °C/sec	1
Denaturation	94	30 sec		40
Annealing/Extension	60	1 min		40
Enzyme Deactivation	98	10 min		1
Hold (optional)	4	Infinite		1

***Table 2**: Cycling conditions for Bio-Rad's C1000 Touch Thermal Cycler**

* Use a heated lid set to 105°C and set the sample volume to 40 µL.

* Modify thermal cycling conditions as required for individual assay.

***WARNING:** using a different ramp rate may impact PCR-amplification and results.*

Data Acquisition and Analysis

1. After thermal cycling, place the sealed 96-well plate in QX200 Dx Droplet Reader
2. Open QX200 software (QuantaSoft™) to set up a new plate layout according to your experimental design.
3. Refer to the QX200 Dx Droplet Reader Manual (Catalog No. 10000044967) for detailed instructions.
4. Designate the following information in the software:
   - Sample Name
   - Experiment type
   - Supermix Type: ddPCR Supermix for Probes (No duTP)
   - Target Names
   - Target Type(s): (Ch1 for FAM and Ch2 for HEX/VIC)
5. Select "Apply" to setup the plate layout, and select "OK" to finish.
6. Upon completion, select "RUN" to begin the droplet reading process (Data Acquisition).
7. After Data Acquisition is complete, analyze and review output.
8. The output reported is copies/ µL of the final 1x ddPCR reaction.

Notes:

Notes:

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For further information, please contact the Bio-Rad office nearest you or visit our website at www.bio-rad.com/diagnostics

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